Engineered fiber-based anisotropic microenvironments for 2D and 3D *in vitro* models of skeletal muscle tissues

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Introduction The functionality of skeletal muscle tissue (SMT) hinges on its hierarchical anisotropic microstructure. In case of large-scale damage, the endogenous repair capability is severely hampered. Failure of traditional 2D cell models in replicating muscle diseases and predicting clinically-relevant drug responses has driven the development of engineered *in vitro* models[1]. Fiber-based scaffolds gained interest as SMT substitutes providing biomimetic geometrical cues. Herein 2D and 3D fibrous *in vitro* models were designed from natural-derived polymers through advanced fabrication techniques.

Experimental methods 2D anisotropic gelatin nanofibers were prepared by electrospinning (A-Gel). C2C12 cells were seeded, and the effect of topographical cues in promoting myotube fusion and maturation was investigated after 10 days. To reproduce more complex skeletal muscle tissue interfaces, fiber-reinforced C2C12-laden bioinks were designed by embedding fragmented A-Gel into an interpenetrating network of Alginate and Gelatin. Multi-scale aligned bioprinted fibrous constructs were produced exploiting shear-stresses.

Results and discussion 2D A-Gel nanofibers showed high myogenic potential inducing the formation of aligned elongated C2C12 myotubes with sarcomere-like organization and myosin-positive myofibers. Then, a novel fibre-reinforced Alginate/Gelatin bioink was designed with improved stability, printability and shape fidelity. Multi-scale aligned 3D architectures produced via micro-extrusion printing supported C2C12 differentiation into muscle fibres arranged anisotropically in mature bundles.

Conclusion Mature myo-bundles with 2D/3D anisotropic organization were obtained by exploiting chemical, topographic and mechanical cues. Such platforms allow to advance toward consistent 2D and 3D *in vitro* models of SMT for preclinical validation, addressing 3Rs principle.

References 1. Zhuang, P. et al.; 193, 108794 (2020).

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